INSTITUT PASTEUR

Microbes and Infection 10 (2008) 726–733

www.elsevier.com/locate/micinf

Original article

Helicobacter hepaticus HHGI1 is a pathogenicity island associated with typhlocolitis in B6.129-IL10^{tm1Cgn} mice

Zhongming Ge [a,1], Torsten Sterzenbach [b,1], Mark T. Whary [a], Barry H. Rickman [a], Arlin B. Rogers [a], Zeli Shen [a], Nancy S. Taylor [a], David B. Schauer [a], Christine Josenhans [b,*,2], Sebastian Suerbaum [b,**,2], James G. Fox [a,***,2]

[a] *Division of Comparative Medicine, Massachusetts Institute of Technology, Building 16-825, 77 Massachusetts Ave., Cambridge, MA 02139, USA*
[b] *Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany*

Received 12 December 2007; accepted 26 March 2008
Available online 4 April 2008

Abstract

Helicobacter hepaticus strain 3B1 (*H. hepaticus*) contains a genomic island of ~71 kb, *HHGI1*, with some of the common features shared among known bacterial pathogenicity islands. In this study, we characterized the pathogenic potential of *HHGI1* by infecting B6.129-IL10^{tm1Cgn} (IL10$^{-/-}$) mice with an isogenic mutant (namely *HhPAId1*) lacking 19 predicted genes within *HHGI1*. In contrast to *H. hepaticus* ($P < 0.001$), *HhPAId1* did not cause typhlocolitis and hyperplasia in IL10$^{-/-}$ mice. Colonization levels of *HhPAId1* were significantly higher in the cecum ($P < 0.007$) and similar in the colon ($P = 0.27$) when compared to *H. hepaticus* by 13 or 16 weeks post inoculation (WPI). The magnitude of the Th1-associated IgG2c response against *HhPAId1* was less than that against *H. hepaticus* ($P < 0.004$). There was no significant difference in Th2-associated IgG1 responses against these two strains. Cecal and colonic mRNA levels of proinflammatory cytokines IFN-γ, TNF-α and IL-17a in the *HhPAId1*-infected mice were significantly lower than those in the *H. hepaticus*-infected mice ($P < 0.05$) at 13 WPI. These results demonstrate that genes in the *HHGI1* contribute to the pathogenicity of *H. hepaticus*, at least in part via up-regulation of proinflammatory mediators IFN-γ, TNF-α and IL-17a.
© 2008 Elsevier Masson SAS. All rights reserved.

Keywords: *H. hepaticus*; Isogenic mutant; Pathogenicity island

1. Introduction

During microbial evolution, many bacterial genomes have acquired blocks of DNA, called "genomic islands", from other organisms by horizontal transfer (reviewed in [1]). These genomic islands can be divided into several subtypes based on their functionality, including ecological islands, saprophytic islands, symbiosis islands and pathogenicity islands (PAI) [1]. PAIs encode one or more virulence-associated factors and often have a G + C content distinct from the rest of the core genome [2]. Bacterial PAIs, such as the well characterized *LEE* in enteropathogenic *Escherichia coli*, *cag* in *Helicobacter pylori* and *SaPI1* in *Staphylococcus aureus*, significantly contribute to the virulence of these pathogens [2].

Helicobacter hepaticus infection leads to chronic hepatitis, hepatocellular carcinoma and typhlocolitis in susceptible mouse strains [3,4]. Recently, it has been demonstrated that this bacterium also contributes to the formation of cholesterol gallstones in C57L/J mice [5], colonic tumors in 129 *Rag2*$^{-/-}$ mice [6], and mammary tumors in *Apc*$^{min/+}$*Rag2*$^{-/-}$ mice [7]. However, our knowledge of virulence factors of this pathogen

* Corresponding author. Tel.: +49 511 532 4348; fax: +49 511 532 4355.
** Corresponding author. Tel.: +49 511 532 6769; fax: +49 511 532 4355.
*** Corresponding author. Tel.: +1 617 253 1735; fax: +1 617 258 5708.
E-mail addresses: josenhans.christine@mh-hannover.de (C. Josenhans), suerbaum.sebastian@mh-hannover.de (S. Suerbaum), jgfox@mit.edu (J.G. Fox).
[1] Authors contributed equally.
[2] Share senior authorship.

1286-4579/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.micinf.2008.03.011

remains limited; the best characterized *H. hepaticus* virulence factor is cytolethal distending toxin (CDT) which is essential for persistent colonization, and plays an important role in the development of *H. hepaticus*-induced typhlocolitis in C57BL/6 *IL10*$^{-/-}$ mice [8] and hepatic dysplasia in male A/JCr mice [9]. A distinct genomic island of ~71 kb (termed *HHGI1*) comprising 70 predicted genes was identified in the completely sequenced genome of *H. hepaticus* strain 3B1 (ATCC51449) [10]. *HHGI1* displays several features of a bacterial PAI, including its relatively low G + C content as compared to the rest of the chromosome and a prophage P4-like integrase, and also like *H. pylori cag*PAI [11], contains several homologs (*VirB10*, *VirB4*, and *VirD4*) of components of the *Agrobacterium tumefaciens* type IV secretion system (T4SS). In addition, the presence of *HHGI1* genes is highly variable among *H. hepaticus* isolates [10], a phenomenon also observed for the *H. pylori cag*PAI [11]. Importantly, male A/JCr mice infected with *H. hepaticus* strains lacking the entire *HHGI1* (MIT 96-1809 isolated from mice originating in the Netherlands) or ~62 out of 71 kb (MIT 96-284 from mice in Germany) developed less severe hepatitis than those infected with *H. hepaticus* 3B1 containing the intact *HHGI1* [12]. These lines of evidence suggested that *HHGI1* is a candidate PAI for *H. hepaticus*. In this study, we generated an isogenic mutant of *H. hepaticus* 3B1, in which a ~21-kb portion of *HHGI1* containing the *VirB10* and *VirB4* homologs was deleted. The effect of this deletion on colonization, pathogenicity and host proinflammatory responses was investigated in B6.129-IL10^{tm1Cgn} mice.

2. Materials and methods

2.1. Helicobacter hepaticus *strains, growth media and conditions*

H. hepaticus strain 3B1 (ATCC 51448) [3] was cultured on blood agar (Remel, Lexington, KY) for 2–3 days under microaerobic conditions (10% H_2, 10% CO_2, 80% N_2). Chloramphenicol (Cm)-resistant *H. hepaticus* mutants were selected on blood agar base supplemented with 10% horse blood and 10 mg/L of Cm.

2.2. Construction of isogenic mutants

In order to construct a mutant of *H. hepaticus* 3B1 where a block of *HHGI1* genes (HH0250–HH0268) was deleted, two regions of approximately 2000 bp were amplified by PCR, one 5′ of gene HH0250 with the primers hepI1P1fw (5′-cgg **ggt acc** TGT GGC TCA TAA GGA GAT CG-3′) and hepI1P1rv (gga *aga tct* ATA CCA TTA TAC CAA GCG ACC) and a second one 3′ of the gene HH0268 with the primers hepI1P2fw (5′-gga *aga tct* TAA CAG GAG TGG TAA CAC GG-3′) and hepI1P2rv (5′-cgg **ggt acc** AGC AGG TGC ATT GCC ATT CC-3′). Capital letters in the primer sequences indicate homologous regions to the genome of *H. hepaticus*, bold letters KpnI-sites, and italic letters BglII-sites. The PCR products were first digested with BglII, then one PCR-product was dephosphorylated and both PCR-products were ligated with each other (Fig. 1). After cleanup, the

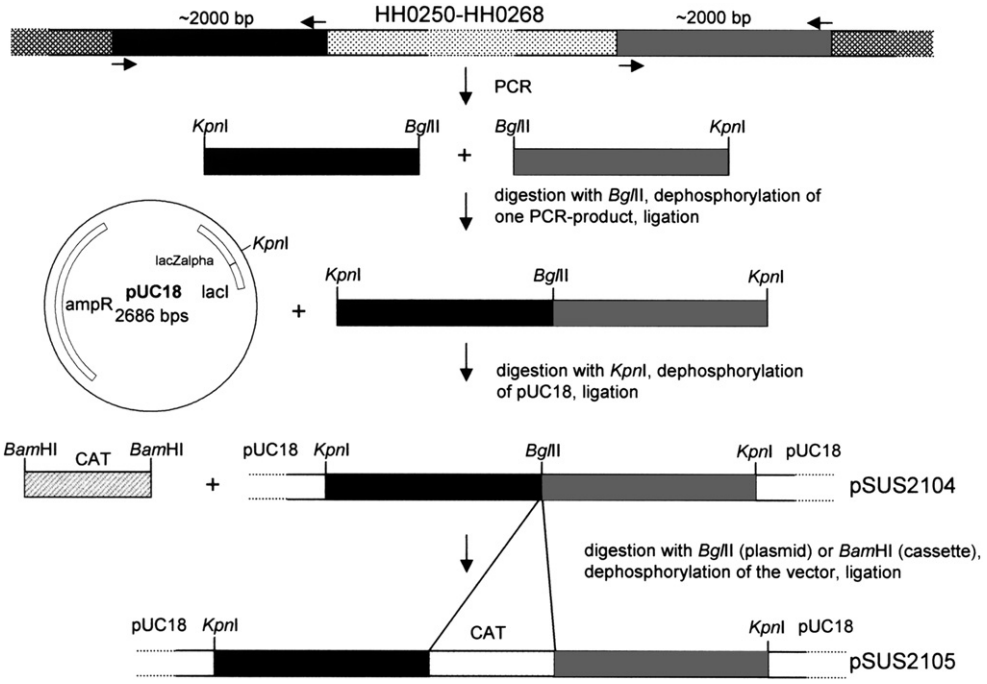

Fig. 1. Construction of plasmid pSUS2105 containing a deletion within *HHGI1*. The hatched region denotes the genetic locus (HH0250–HH0268, ~21.7 kb) that was replaced with the *Campylobacter coli* chloramphemicol acetyltransferase gene (*CAT*) [13]. HH0252 and HH0246 in the deleted island segment display sequence similarity to *Agrobacterium tumefaciens VirB10* and *VirB4*, respectively [10].

Download English Version:

https://daneshyari.com/en/article/3415985

Download Persian Version:

https://daneshyari.com/article/3415985

Daneshyari.com